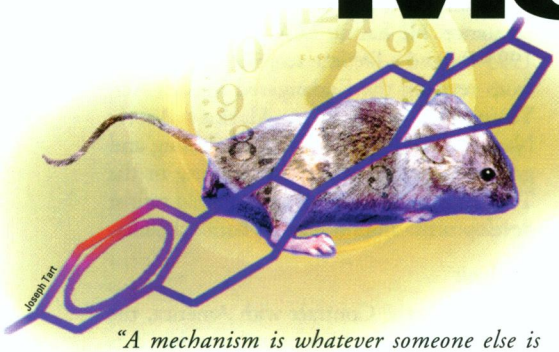
Mouse or Molecule?

Mechanism-based Toxicology in Cancer Risk Assessment



"A mechanism is whatever someone else is working on at a level lower than I am."

—Anonymous toxicologist

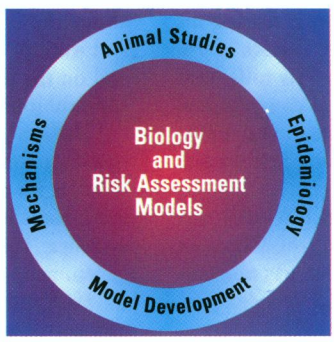
Cancer is a set of diseases characterized by uncontrolled cell growth. It is thought to be a complex process involving multiple steps, any of which may be initiated, altered, and otherwise affected by exposure to chemical carcinogens in the environment. Although an understanding of the process remains incomplete, recent gains in the knowledge of the mechanisms of action of carcinogens both in experimental animals and humans may help refine current risk assessment methodology for identifying and quantifying cancer risks associated with chemical exposure.

For regulatory agencies such as the EPA, the FDA, and the Occupational Safety and Health Administration (OSHA), a certain urgency exists to improve cancer risk assessment. Along with continued concern from the public about the safety of environmental chemicals and tremendous pressure from industry to provide more solid scientific rationales for specific regulatory decisions, there are the numbers: of the 70,000 substances in commerce, adequate toxicological data are available for only 10–20%. And of the 50 top-production chemicals in the United States (which total nearly 700 billion pounds per year), more than two-thirds have yet to be evaluated for carcinogenicity in animals.

These pressures, of course, also have an impact on that portion of the scientific community charged with providing public policy makers with the most complete information on the environmental components of human disease and on the biological mechanisms by which these diseases occur. In terms of cancer risk assessment, the problem is compounded by time and money. The conventional 2-year rodent bioassay, which typically forms the basis of cancer risk assessment, costs between $2 and $4 million and requires 4–6 years to complete. "At present, with current resources, we can test only 10 to 15 chemicals by this approach each year," says George Lucier, director of the Environmental Toxicology Program at the NIEHS.

Another important issue is uncertainty. Cancer risk assessment of chemical exposure relies heavily on tumor data from animal carcinogenicity bioassays conducted at high doses. Positive bioassay findings are frequently followed by mathematical extrapolation to the much lower exposure levels anticipated for environmental exposures of humans.

Along the way to a carcinogenicity assessment, and in the absence of knowlege that demonstrates otherwise, certain conservative inferences or "defaults" are assumed such as the conservation of biological processes among species, the inference of similar susceptibility between animals and humans; the assumption that susceptibilities within a population do not differ by age, gender, or genetics; and the assumption of low-dose linearity—that chemicals act like radiation at low doses to induce cancer, and that a single mutational change can result in adverse effects down the road. And so, when actual or more accurate data are either unavailable, incomplete, or inadequate, use of defaults add their own varying degrees of uncertainty regarding the carcinogenicity of a particular chemical. Thus, says Lucier, regulatory agencies are often forced to make decisions on chemicals and safe exposure levels without an adequate science base.



Modes of Action

"We are charged with decreased use of animals, charged with developing alternatives for screening chemicals more rapidly and to set priorities for further testing," says J. Carl Barrett, head of NIEHS's Environmental Carcinogenesis Program. "Mechanism-based toxicology presents one type of alternative." According to Barrett, mechanism-based toxicology (MBT) involves "the use of knowledge of mechanisms both of disease process and of chemical effects to better predict the toxicological potential of chemicals, to estimate risk at low doses, to extrapolate between species, and to quantitate interindividual differences in response."

Support for the use of MBT data can be found in EPA's proposed revisions to its *Guidelines for Carcinogen Risk Assessment* (August 1994). In its draft document, the agency leaves room for inclusion of more biologically based data as a means of reducing the uncertainty associated with extrapolation of risk. While acknowledging that the mechanisms by which any chemical causes cancer may never be completely detailed, scientists involved in formulating the proposed EPA revisions generally agreed that this should not preclude the use of scientifically supportable "mode of action data" in the risk assessment process.

Barrett points to three fundamental modes or mechanisms of action by which chemically induced cancer can arise: heritable mutations in critical target genes, heritable epigenetic changes in cellular phenotype, and clonal evolution or expansion of cells influencing the probability of subsequent mutations occurring either spontaneously or through exogenous exposure. An example, explains Barrett, is diethylstilbestrol (DES) exposure *in utero*. Developmentally timed exposure of this synthetic

estrogenic compound can change the tissue differentiation pattern throughout the lifetime. "If you give DES during the first five days of development, adult animals have a totally different pattern, a different differentiation, than nonexposed animals. So there are critical periods in development where exposures can change the progression of growth and control of cells that may lead to the cancer process," says Barrett. "These are not mutually exclusive. A chemical can exert multiple mechanisms or modes of action."

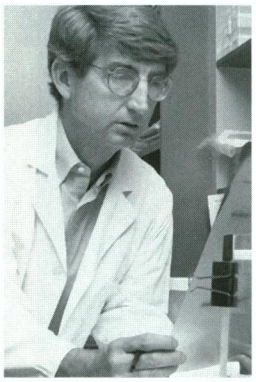
Advantages

As Barrett points out, advantages of MBT are that it allows many more substances or chemicals to be tested for biological activities, provides an alternative to the use of animals, and provides more efficient design of toxicology studies. "For example, if you know a chemical exerts an impact on cell–cell communication, then you might look at its impact on cancer or noncancer endpoints," Barrett says.

MBT may also be applied to testing the validity of certain default assumptions; for example, the conservation of biological processes, including mechanisms. "If you look at how the steroid hormone receptor functions, it doesn't matter if it's a yeast or a man; it functions the same way," says toxicologist Linda Birnbaum of EPA's Health Effects Research Laboratory. "And I think if you understand how an effect or part of an effect is brought about, you can look at whether those mechanisms are working in man as well as in experimental animals." Birnbaum adds: "Sometimes there are much shorter assays that demonstrate carcinogenic potential. Maybe by applying mechanistic approaches one can understand under what circumstances that potential is realized."

In the initial stages of risk assessment, MBT can be used to more rapidly screen chemicals and to set priorities for further studies. In terms of mutational activity, for example, current knowledge of critical suppressor genes, such as p53, might be exploited to understand the carcinogenic mechanisms of specific chemicals. Another example is receptor-mediated pathobiology, the knowledge that some chemicals, such as dioxin and estrogens, seem to cause diseases by interacting or binding with specific receptors. Molecular proteins such as the Ah receptor may help predict the toxicological impact of other chemicals. And in terms of structure–activity relationships (SAR), the knowledge that certain types of chemicals do or do not share structural or biological properties associated with mechanisms critical to carcinogenesis can help strengthen or weaken concern about an agent's carcinogenicity. Mutational and SAR analysis as well as knowledge of receptor-mediated activity may also offer a rationale for reasonably assuming hazard and may preclude costly and time-consuming bioassays.

Another use of MBT in cancer risk assessment is in determining dose–response relationships for chemical effects at low doses. Dose–response assessment refers to the process of estimating the relationship of dose of a substance to degree of effect. MBT can be used to explore and identify mutational activity, receptor-mediated effects, pharmacodynamics, and pharmacokinetics.



Robert Wood Johnson Med.

Michael Gallo—We have to take a hard look at receptor-mediated mechanisms.

Michael Gallo, director of the NIEHS Center of Excellence at New Jersey's Robert Wood Johnson Medical School, says he sees a greater emphasis on the role played by receptor-mediated mechanisms involved in carcinogenesis, particularly the hormonal component. "We now know that the receptor-mediated mechanisms may be the classic of all cancer promoters. The receptors we're studying are involved in growth regulation—the Ah receptor, the estrogen receptor, the epidermal growth factor receptor, the thyroid hormone receptor. We have to take a hard look at them." Thus, it follows that dose–response assessment for an effect other than mutagenesis or tumor incidence may be useful for assessing potential environmental carcinogens. If carcinogenic effects are secondary to precursor molecular effects, such as disruption of hormonal activity, such precursor events may be more relevant than tumor incidence for risk assessment. Gallo says a major area of concern today is the role played by hormonal or endocrine disruptors in the environment. "If we can show that disruption or modification of a hormone is in fact an effector for cancer, then there are secondary or tertiary mechanisms involved."

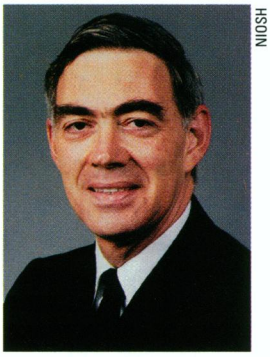
Measurement of biochemical or molecular events following chemical exposure has also sparked considerable interest in the use of biomarkers in risk assessment. MBT may be useful in evaluating the quantitative and qualitative relationships of these markers to toxic effects. At the recent NTP Workshop on Mechanism-based Toxicology in Cancer Risk Assessment, a dose–response work group considered the value of biomarkers for risk assessment. They said some biomarkers may help determine exposure to a carcinogen and its effect. For example, biomarkers reflect gene mutations directly related to carcinogenesis or to alterations in gene expression. Comparisons of animal model and human molecular biomarkers were viewed as potentially useful for establishing interspecies dosimetry and susceptibility.

Another NTP workshop subgroup concluded that it would be useful and appropriate to apply mechanistic information to assessing the relevance of the rodent bioassay for humans. Participants pointed out that the collection of relevant mechanistic data can either support the interspecies extrapolation default or could otherwise provide a

NIEHS

Meeting on mechanisms. A recent meeting on mechanism-based toxicology brought together leaders in environmental health. (left to right) George Lucier, Lynn Goldman, EPA assistant administrator for prevention, pesticides and toxic substances, NIEHS Director Kenneth Olden, and J. Carl Barrett.

rationale for revising it. They also concluded that mechanistic information could be used prospectively in the design of chronic rodent bioassays as an aid in selecting route of administration, dosing, species selection, and endpoints. If, for example, the data indicated compound-induced cell proliferation or cytotoxicity, this would provide a basis for monitoring those endpoints in the bioassay. The workshop subgroup saw biomarkers of susceptibility, such as those indicative of polymorphisms of metabolism, detoxication, and DNA repair, as presenting "a promising opportunity for interindividual and interspecies extrapolation," as well as having possible utility for evaluating age and/or sex-related differences in human susceptibility.



NIOSH

Brian Hardin—Scientific data can be used to delay action.

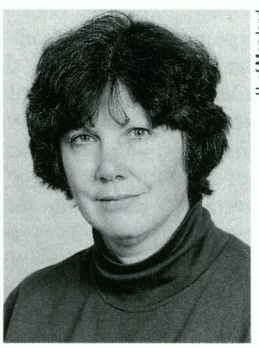
What is Enough?

Mechanism-based toxicology appears to offer tremendous potential for cancer risk assessment, but how much evidence is enough? As Barrett points out, in spite of MBT's potential, what is needed is a comfort level with some sort of minimum data for risk assessment decisions. Toxicologist Ellen Silbergeld of the University of Maryland, agrees. "It is important to come up with some understanding as to what is the minimum amount of data that would allow both scientists and regulators to proceed together with some degree of confidence, with some ability to communicate to the public, the nature and extent of risk." She cautions that unless some limits are set, "We are in danger of being attracted into the very gray area between risk assessment-related research and basic biology, which is the endless quest for knowledge but may not be exactly consonant with the needs as well as the resources to make informed and practical decisions. I think of an analogy to clinical medicine, and I am aware now that as I teach medical students that they don't have to understand everything about molecular biology and disease process in order to make a clinical decision. It seems to me we've been remiss in establishing that same type of strategic outlook in how we bring science into the regulatory process."

For Brian Hardin, senior scientist in the Office of the Director at NIOSH, the issue can be viewed from another perspective. "Pursuit of more and better scientific data can be used very effectively by forces whose interests are served by avoiding action, by delaying action . . . 'paralysis by analysis'." He says these forces can make skillful and plausible appeals for more and better science before some government intervention is allowed that would disturb the established order, perhaps at the expense of public health. "Whose risk is being minimized?" he asks.

Hardin, along with other scientists, also expresses concern that resources gained for mechanistic research will come at the expense of whole-animal bioassays and epidemiologic studies. "Those sorts of studies provide the most convincing and most powerful tools we have today for protecting human health. Despite the faith we all have in mechanistic work, I predict it will be many, many years before it is possible to regulate any chemical in commerce as a carcinogen in the absence of epidemiologic or animal evidence of carcinogenicity," Hardin says.

Birnbaum points out that conventional long-term bioassays may still be controversial after their completion. She says the use of short-term MBT studies that are highly predictive of a compound's likelihood of carcinogenicity could be used to support two-year bioassay findings. "That in fact could result in agreement that the compound is a bad actor and should not be used. In that sense we would have done a good job of protecting the public health."



U. of Maryland

Ellen Silbergeld—There needs to be an understanding about the minimum amount of data needed for risk assessment.

Recommended by Birnbaum and others is a more iterative and integrative approach to data collection for risk assessment purposes. At the NTP workshop, Silbergeld reminded participants that the bioassay is an extremely rich source of information and offers the only lifetime surveillance opportunity of an animal model. She recommends expanding the bioassay to provide more mechanistic information as it is proceeding. "That may allow you to stop at certain times, evaluate where you are, but at the same time preserve an established structure and possibly be more economical overall because you'll be able to eliminate as you go along," Silbergeld adds. "I don't think the problem here is including mechanistic information. The problem here is making justifiable selection among an enormous array of potential mechanistic approaches that could be applied."

Few would argue that increased use of mechanism-based toxicology should mean severely diminished use of the conventional bioassay. Rather, MBT should be used in addition to current testing strategies, not as a substitute for them, and study designs should be developed to provide the information needed for the best-informed risk assessments.

Today, several key impediments exist to the use of mechanistic data in risk assessment for cancer: comfort among scientists and regulators with the status quo; constraint by regulatory legislation; concern with the implications for making costly decisions. It has been said that science needs to evolve to increase its own confidence in mechanistically based predictions. For this work to continue, it is imperative that confidence also be increased among legislators, regulators, and the public.

Uses of MBT

- To more rapidly screen chemicals and set priorites for further studies
- As a basis for reasonably assuming hazard (rebuttable presumption)
- To determine quantitative dose–response relationships
- To understand species, strain, and individual differences in susceptibility
- For species extrapolations
- For more efficient experimental design

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Suggested Reading

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